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The Contents of Case 09870379

Qnum	Query	DB Name	Thesaurus	Operator	Plural
Q1	pi3 kinase inhibitor	USPT	ASSIGNEE	ADJ	YES
Q2	Q1 and cancer	USPT	ASSIGNEE	ADJ	YES
Q3	wortmannin	USPT	ASSIGNEE	ADJ	YES
Q4	Q3 and angiogenesis	USPT	ASSIGNEE	ADJ	YES
Q5	ly294002	USPT	ASSIGNEE	ADJ	YES
Q6	Q5 and cancer	USPT	ASSIGNEE	ADJ	YES
Q7	Q6 and angiogenesis	USPT	ASSIGNEE	ADJ	YES

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End of Result Set

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L1: Entry 13 of 13 File: USPT Mar 16, 1999

DOCUMENT-IDENTIFIER: US 5882910 A
TITLE: Lipid kinase

Detailed Description Text (16):
To determine the PI 3-kinase activity of p110.delta. b, 5.mu.l of immunoprecipitated p110.delta. was mixed with 1 .mu.l of PI/EGTA and incubated at room temperature for 10 minutes [PI/EGTA is 10 mg/ml PI (Sigma) in CHCl.sub.3, which has been dried under a vacuum, resuspended in 20 mg/ml DMSO in the presence or absence of various concentrations of the PI3 kinase inhibitor wortmannin and diluted 1:10 in 5 mM EGTA] and added to 1 .mu.l 10.times. HM buffer (200 mM HEPES pH7.2, 50 mM MnCl.sub.2), 0.5 .mu.l .gamma..sup.32 PATP (10 mCi/ml-300 Ci/mmol), 1 .mu.l 100 .mu.M ATP, and 1.5 .mu.l H.sub.2 O and incubated at 30.degree. C. for 15 minutes. The reactions were terminated by addition of 100 .mu.l 1M HCl. Lipids were extracted with 200 .mu.l CHCl.sub.3 /MeOH (1:1) by vortexing for 1 minute followed by centrifugation at 16,000.times.g for 2 minutes at room temperature. The lipids were further extracted with 80 .mu.l 1M HCl/MeOH (1:1) by vortexing for 1 minute, followed by centrifugation at 16,000.times.g for 2 minutes at room temperature. The lipids were dried under vacuum, resuspended in 10 .mu.l CHCl.sub.3 /MeOH (1:1) and spotted 2 cm from the bottom of a dry Silica gel 60 chromatography plate (VWR) that had been pre-impregnated with 1% K.sub.2 C.sub.2 O.sub.4 in H.sub.2 O. 250 .mu.g of crude phosphoinositides (Sigma) were spotted as markers. The products were resolved by chromatography for 2 hours in CHCl.sub.3 /MeOH/4N NH.sub.4 OH (9:7:2), allowed to dry and placed in an Iodine vapor tank for 5 minutes in order to visualize the crude standards. The position of the standards was marked with a pencil and the plate was autoradiographed.

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L4: Entry 3 of 13 File: USPT Sep 10, 2002

US-PAT-NO: 6448054
DOCUMENT-IDENTIFIER: US 6448054 B1

TITLE: Purposeful movement of human migratory cells away from an agent source

DATE-ISSUED: September 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Poznansky; Mark C.	Charlestown	MA		
Luster; Andrew T.	Wellesley	MA		
Scadden; David T.	Weston	MA		

US-CL-CURRENT: 424/184.1; 424/85.1

CLAIMS:

We claim:

1. A method of repelling immune cells from a specific site in a subject, comprising: locally administering to a specific site in a subject in need of such treatment a fugetactic agent in an amount effective to repel immune cells from the specific site in the subject.

2. The method of claim 1, wherein the specific site is a site of inflammation.

3. The method of claim 2, further comprising co-administering a non-fugetactic agent that inhibits migration of immune cells to the site of inflammation in the subject.

4. The method of claim 3, wherein the non-fugetactic agent is an antiinflammatory agent.

5. The method of claim 1, wherein the subject has an autoimmune disease.

6. The method of claim 5, wherein the autoimmune disease is rheumatoid arthritis, uveitis, insulin-dependent diabetes mellitus, hemolytic anemias, rheumatic fever, Crohn's disease, Guillain-Barre syndrome, psoriasis, thyroiditis, Graves' disease, myasthenia gravis, glomerulonephritis, autoimmune hepatitis, or systemic lupus erythematosus.

7. The method of claim 1, wherein the subject has an abscess, a transplant, an implant, atherosclerosis, or myocarditis.

8. The method according to claim 1, wherein the fugetactic agent is SDF-1.alpha. at a concentration higher than about 1 .mu.g/ml, non-diluted thymic stromal-cell-derived medium, concentrated thymic stromal-cell-derived medium, or a thymic stromal-cell-derived polypeptide factor.

9. The method of claim 8, wherein the thymic stromal-cell-derived polypeptidc factor mediates its repellent effects through a G-protein transduction pathway.

10. The method of claim 9, wherein the thymic stromal-cell-derived polypeptide factor is not genistein inhibited.

11. The method of claim 9, wherein the thymic stromal-cell-derived polypeptide factor is wortmannin inhibited.

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L4: Entry 4 of 13 File: USPT Sep 3, 2002

DOCUMENT-IDENTIFIER: US 6444233 B1
TITLE: Triterpene compositions and methods for use thereof

Brief Summary Text (57):
Another important aspect of this invention is a method of regulating angiogenesis in a mammal comprising administering to the mammal a therapeutically effective amount of the pharmaceutical compositions described. The method may be when the mammal is a human.

Brief Summary Text (59):
In addition to providing methods of preventing or treating cancer with the compounds of the invention, the inventors have provided a number of other uses for the compounds of the invention. In particular, the compounds of the invention may be used as solvents, antioxidants, anti-fungal and anti-viral agents, piscicides or molluscicides, contraceptives, antihelmintics, angiogenesis regulators, UV-protectants, expectorants, diuretics, anti-inflammatory agents, regulators of cholesterol metabolism, cardiovascular effectors, anti-ulcer agents, analgesics, sedatives, immunomodulators, antipyretics, as agents for decreasing capillary fragility, as agents to combat the effects of aging, as agents for increasing skin collagen, as agents for enhancing penile function and as agents for improving cognition and memory.

Drawing Description Text (23):
FIG. 21: Lipid kinase assay demonstrating inhibition of PI3-Kinase by UA-BRF-004-DELEP-F035 and wortmannin.

Drawing Description Text (24):
FIG. 22: SDS-PAGE gel analyzed by western-ECL using phospho-specific AKT and total AKT antibody. Post treatment of cells with 1 and 2 .mu.g/ml of UA-BRF-004-DELEP-F035 caused a marked inhibition of AKT phosphorylation (active AKT), which was similar to a 2 hour treatment of cells with 1 .mu.M of wortmannin.

Detailed Description Text (334):
The inventors specifically contemplate the use of the compounds of this invention for a range of applications in addition to the treatment or prevention of cancer. In particular, the inventors contemplate the use of the triterpene compounds of the invention as solvents, anti-fungal and anti-viral agents, piscicides or molluscicides, contraceptives, antihelmintics, UV-protectants, expectorants, diuretics, anti-inflammatory agents, regulators of cholesterol metabolism, cardiovascular effectors, anti-ulcer agents, analgesics, sedatives, immunomodulators, antipyretics, angiogenesis regulators, as agents for decreasing capillary fragility, as agents to combat the effects of aging, and as agents for improving cognition and memory.

Detailed Description Text (335):
The compounds of this invention have a role in the regulation of angiogenesis. Angiogenesis or neovascularization is defined as the growth of new blood vessels. Tumors and cancers induce angiogenesis to provide a life-line for oxygen and nutrients for the tumor to thrive. The development of new blood vessels also provide exits for malignant cancer cells to spread to other parts of the body. Angiogenesis inhibition therefore benefits cancer patients. On the other hand, angiogenesis is required at times such as wound healing. These wounds can be external wounds or internal organ wounds that result from accidents, burns, injury and surgery. Thus, agents that promote angiogenesis have a great potential for use in therapy for wound healing.

Detailed Description Text (381):
In light of the present disclosure, one could utilize screening assays for the identification of compounds having essentially the same chemical characteristics and biological activity as those described herein. In particular, the present disclosure would allow one to employ assays for biologically active triterpene glycosides from those plants closely related to Acacia victoriae, for example, members of the genus Acacia. These assays may make use of a variety of different formats and may depend on the kind of "activity" for which the screen is being conducted. Preferred assays comprise those directed to screening for anti-tumor activity, such as described herein for extracts from Acacia victoriae. As used herein, "anti-tumor activity" refers to the inhibition in tumor cells of cell-to-cell signaling, growth, metastasis, cell division, cell migration, soft agar colony formation, contact

inhibition, invasiveness, angiogenesis, tumor progression or other malignant phenotype or the induction of apoptosis. Particularly contemplated are functional assays which include measures of the use of the compounds of the invention as anti-fungal and anti-viral agents, piscicides or molluscicides, contaceptives, anthelmintics, UV-protectants, expectorants, diuretics, anti-inflammatory agents, regulators of cholesterol metabolism, cardiovascular effectors, anti-ulcer agents, analgesics, sedatives, immunomodulators, antipyretics, regulators of angiogenesis, and as agents for decreasing capillary fragility. Such assays will be well known to those of skill in the art in light of the instant disclosure. As well as in vitro and in vivo direct assays for activity, these assays may include measures of inhibition of binding to a substrate, ligand, receptor or other binding partner by a compound of the invention.

Detailed Description Text (568):
Angiogenesis or neovascularization is a process by which cells are recruited by factor(s) produced by a tumor to provide the tumor with a nourishing vascular system. Inhibiting angiogenesis inhibits tumor expansion by limiting blood supply to the tumor. This function was examined using a bovine capillary endothelial cell proliferation assay on cells treated with Fraction 35 (UA-BRF-004-DELEP-F035). The assay was carried out as follows: bovine capillary endothelial cells were obtained and grown using standard procedures (Folkman et al., 1979). The cells were washed with PBS and dispersed in a 0.05% trypsin solution. A cell suspension (25,000 cells/ml) was made with DMEM+10% BCS+1% GPS, plated onto gelatinized 24 well culture plates (0.5 ml/well) and the suspension incubated for 24 h at 37.degree. C. The media was replaced with 0.25 ml of DMEM+5% BCS+1% GPS and different concentrations of UA-BRF-004-DELEP-F035 applied. After a 20 min incubation, media and bFGF were added to obtain a final volume of 0.5 ml DMEM+5% BCS+1% GPS+1 ng/ml bFGF. After 72 h the cells were dispersed in trypsin, resuspended in Hematall (Fischer Scientific, Pittsburg, Pa.) and counted by coulter counter (O'Reilly et al., 1997).

Detailed Description Text (569):
The results of the assay demonstrated significant inhibition of endothelial cell proliferation with or without basic fibroblast growth factor (FIG. 5). These results demonstrate that the active components of the plant extract are potent inhibitors of endothelial cell proliferation, which is often a predictor of in vivo suppression of angiogenesis. In addition, the fraction had no effect on migration of capillary endothelial cell, suggesting lack of toxicity to normal cells (FIG. 6).

Detailed Description Text (576):
In order to further elucidate the molecular targets of the active components of the Acacia victoriae plant extract, a study was conducted on the effect of F035 on phosphatidylinositol 3-kinase (PI3-kinase) activity, as well on AKT (protein kinase B, a serine-threonine kinase) activity, a downstream effector of PI3-kinase. P13-kinase is an enzyme which is implicated in growth factor signal transduction by associating with receptor and non-receptor tyrosine kinases. There are two known PI3-kinase inhibitors: wortmannin, a fungal metabolite, and LY294002, a synthetic compound which is structurally similar to the plant bioflavonoid quercetin.

Detailed Description Text (577):
The assay was carried out as follows: Jurkat cells (1.times.10.sup.7) were starved overnight and exposed to different concentrations (1-8 .mu.g/ml depending upon the cells line) of F035 for various times (2-16 h) at 37.degree. C. After different time points, the cells were collected and washed with PBS at 2000 rpm for 10 min. The cells were lysed in 1% NP-40 lysis buffer for 30 min at 4.degree. C. and the lysates isolated by centrifugation for 5 min at 15,000 rpm at 4.degree. C. In order to conduct immunoprecipitation of PI3-kinase, 5 .mu.l of rabbit anti-p85 antibody (tyrosine kinase receptor adapter protein; Upstate Biotechnology Inc.) was incubated with 1 ml of cell lysate for 90 min at 4.degree. C. The immune complexes were isolated on 100 .mu.l of 20% Protein A-Sepharose beads for an additional 90 min at 4.degree. C. The immunoprecipitates were washed sequentially in a) PBS, 100 mM Na3VO4, 1% Triton-X100; b) 100 mM Tris, pH 7.6, 0.5 LiCl, 100 mM Na3VO4; c) 100 mM Tris, pH 7.6, 100 mM NaCl, 1 mM EDTA, 100 mM Na3VO4; and d) 20 mM Hepes pH 7.5, 50 mM NaCl, 5 mM EDTA, 30 mM NaPPi, 200 mM Na3VO4, 1 mM PMSF, 0.03% Triton X-100. Immunoprecipitates were then resuspended in 30 .mu.l of kinase reaction buffer (33 mM Tris, pH 7.6, 125 mM NaCl, 15 mM MgCl.sub.2, 200 mM of adenosine, 20 mM ATP, 30 .mu.Ci [g-32P] ATP). Phosphatidyle inositol (PI; 50 .mu.I) was dried under nitrogen gas and resuspended in 20 mM HEPES, pH 7.5 at 2 mg/ml and sonicated on ice for 10 min. The PI3-kinase reaction was initiated by addition of 10 .mu.l of the PI suspension and 10 .mu.l of gamma-ATP. The reaction was allowed to proceed for 30 min at room temp, followed by termination of the reaction by addition of 100 .mu.l of 1N HCl. Lipids were extracted with 600 .mu.l chloroform: methanol (1:1) and resolved on silica gels (G60) by thin-layer chromatography (TLC) in chloroform : methanol: NH.sub.4 OH:H.sub.2 O (60:47:2:11.3). Radio labeled phosphatidylinositol phosphate was visualized by autoradiography and inhibition was quantitated by storm system (Okada et al., 1994; Vlahos et al., 1994). The results (FIG. 21) indicate that 2 and 6 hours post-treatment with F035 (4 .mu.g/ml) there was an inhibition of PI3-kinase activity. Similarly, when cells were exposed to 2 .mu.g/ml of F035 for 15 h, a 95% inhibition was observed, similar to wortmannin (a fungal metabolite and known inhibitor of PI3-kinase) in Jurkat cells.

Detailed Description Text (578):
Next, the effect of F035 on AKT, a downstream effector of PI3-kinase, was studied. AKT, also known as protein kinase B, is a cellular homologue of viral oncogene v-AKT and is activated by number of growth factors and functions in a pathway involving PI3-K activation, which is sensitive to wortmannin. AKT codes for serine-threonine protein kinase, which has been shown to be

amplified in 12.1% of ovarian carcinomas and 2.8% of breast cancers. AKT is involved in an anti-apoptotic pathway through phosphorylation of Bad, an anti-apoptotic molecule. Ovarian cancer patients with AKT alterations appear to have poor prognosis (Bellacosa et al., 1995). AKT has been shown to actively block apoptosis, partly by activation of p70S6 kinase (Kennedy et al., 1997). p70S6 kinase is a mitogen activated serine-threonine protein kinase required for cell growth and G1 cell cycle progression (Chou and Blenis, 1996). The activity of p70S6 kinase is controlled by multiple phosphorylation events located within catalytic and pseudosubstrate region (Cheatham et al., 1995; Weng et al., 1995).

Detailed Description Text (579):
The effect of F035 on phosphorylation of AKT was analyzed as follows. Jurkat cells (5.times.10.sup.6) were serum starved and exposed to F035 for 15 h and 2 h with wortmannin at 37.degree. C. The cells were either induced with cd3XL (cd3 crosslink) or left uninduced for 10 min at 37.degree. C. and lysed in AKT lysis buffer and the proteins were resolved on 8% SDS-PAGE gels and analyzed by western-ECL using phospho-specific AKT (Ser 473; New England Biolabs) and total AKT antibody. An assay of the effect of F035 on p70S6 kinase can be carried out similarly, but using a Phosphoplus p70S6 kinase antibody kit (New England Biolabs) for analysis of p70S6 kinase (Ser 411, thr421/ser424) phosphorylation. The results of the AKT analysis (FIG. 22), demonstrated that cd3 crosslink induces phospho AKT slightly. Post treatment of cells with 1 and 2 .mu.g/ml of F035 caused a marked inhibition of AKT phosphorylation (active AKT), which is similar to a 2 h treatment of cells with 1 .mu.M of wortmannin. There was, however, no change in the expression of total AKT. Similar inhibition of AKT phosphorylation was also demonstrated using ovarian cancer cells OVCAR-3 and C-2 (HEY variant), and with Jurkat cells treated with 2-4 .mu.g/ml of F094. These findings demonstrate that F035 inhibits the phosphorylation of AKT in Jurkat cells and ovarian cancer cells. This is significant given that the PI3 kinase/AKT signaling pathway has been shown to deliver an anti-apoptotic signal (Kennedy et al., 1997). The results suggest F035 and F094 is mediating apoptosis of tumor cells through the suppression of the PI3-K signaling pathway.

Detailed Description Text (655):
The serum starved Jurkat cells were treated with 2 .mu.g/ml of F035 for 2-15 h or 0.5 h with wortmannin at 37.degree. C. PI 3-kinase activity was determined as described (Whitman et al., 1985; Royal and Park, 1995). PI3-kinase was immunoprecipitated from 1 mg of cellular protein using 5 .mu.l rabbit anti p85 antiserum at 4.degree. C. for 90 min. The immune complexes were collected on 20% protein A-sepharose beads for 90 min at 4.degree. C. Next the immunoprecipitates were resuspended in 30 .mu.l of kinase reaction buffer (33 mM Tris, pH 7.6, 125 mM NaCl, 15 mM MgCl.sub.2, 200 mM of adenosine, 20 mM ATP, 30 uCi [g-32P] adenosine triphosphate ATP). The PI3-kinase reaction was initiated by addition of 10 .mu.l of the PI suspension and 10 .mu.l of gamma-ATP and allowed to proceed for 30 min at room temp. Adding 100 .mu.l of 1 N HCl terminated the reaction. Lipids were extracted from the reaction mixture with chloroform: methanol (1:1) and resolved by thin layer chromatography (TLC) in chloroform: methanol: NH.sub.4 OH: H.sub.2 O (60:47:2:11.3) on silica gel G60 plates. Radio labeled phosphatidylinositol (PI) phosphate was visualized by autoradiography and inhibition was quantitated using a Storm 860 system (Molecular Dynamics).

Detailed Description Text (676):
To study the molecular target(s) of F035, the inventors investigated the PI3-kinase signaling pathway. The results of immunoprecipitation with anti-p85 antibody (adapter protein) probe and subsequent lipid kinase assay showed that F035 inhibits the activity of PI3-kinase in Jurkat cells. FIG. 45A demonstrates about 50-70% inhibition of PI3-kinase activity with in 2 hr post treatment with F035. By 6 hr 92-95% inhibition of PI3-kinase activity was observed which persisted up to 15 hr post treatment. Wortmannin [1 .mu.M, 30 min post treatment], a known PI3-kinase inhibitor showed similar inhibition of enzyme activity in Jurkat cells (FIG. 45A).

Detailed Description Text (679):
The inventors determined the effect of F035 and pure extracts on AKT, a serine threonine kinase and a downstream effector of the PI3-kinase signaling pathway. In contrast to the rapid inhibition of PI3-kinase activity, inhibition of AKT phosphorylation did not occur till 15 hr post treatment. Treatment of Jurkat cells with F035 (2 ml) for 15 hr led to decreased phosphorylation of AKT. However, this treatment also led to lowered levels of total AKT protein as can be seen in FIG. 45B. The inventors confirmed the inhibition of AKT activity with pure triterpene glycosides. Pure triterpene glycosides D1 & G1 (2 .mu.g/ml) also inhibited AKT phosphorylation and total AKT protein expression. (FIG. 45B). Treatment of Jurkat cells with LY 294002 and wortmannin (known PI3-kinase inhibitors) showed inhibition of AKT phosphorylation.

Detailed Description Text (682):
In order to further study the mediators of apoptotic pathway, the inventors evaluated the effects of F035, D1 and G1 on the transcription factor NF-.kappa.B which has been shown to be involved in apoptosis. The results in FIG. 46A show that in Jurkat cells, F035 inhibited the TNF-dependent activation of NF-.kappa.B in a dose dependent manner. Untreated cells and cells treated with F035 alone showed no activation of NF-.kappa.B. The inventors also confirmed these results with pure extracts D1 and G1. Pretreatment of cells with 2 ml of G1 and D1 resulted in 54% and 87% decrease in NF-.kappa.B levels respectively (FIG. 46B). Cells treated with D1 or G1 alone showed no activation of NF-.kappa.B (FIG. 4613). Since recently PI3-kinase has been shown to regulate NF-.kappa.B, pretreatment of cells with wortmannin (1 .mu.M) resulted in almost total inhibition of TNF-induced

NF-.kappa.B.

Detailed Description Text (685):
As the transcription of iNOS is regulated by NF-.kappa.B, the inventors investigated the effect of F035 on the induction of iNOS. In U-937 cells which were differentiated into macrophages the inventors induced iNOS in response to LPS (FIG. 46C). Pretreatment of these cells with F035 (1 .mu.g/ml) totally blocked the induction of iNOS. Wortmannin also had a similar effect on LPS induced iNOS in these cells.

Other Reference Publication (56):
Norman, "Studies on the mechanism of phosphatidylinositol 3-kinase inhibition by wortmannin and related analogs," J. Med. Chem., 39:1106-1111, 1996.

Other Reference Publication (58):
Okada et al., "Blockage of chemotactic peptide-induced stimulation of neutrophils by wortmannin as a result of selective inhibition of phosphatidylinositol 3-kinase,"J. Bio. Chem., 269:3563-3567, 1994.

L4: Entry 4 of 13 File: USPT Sep 3, 2002

DOCUMENT-IDENTIFIER: US 6444233 B1
TITLE: Triterpene compositions and methods for use thereof

Brief Summary Text (57):
Another important aspect of this invention is a method of regulating angiogenesis in a mammal comprising administering to the mammal a therapeutically effective amount of the pharmaceutical compositions described. The method may be when the mammal is a human.

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In light of the present disclosure, one could utilize screening assays for the identification of compounds having essentially the same chemical characteristics and biological activity as those described herein. In particular, the present disclosure would allow one to employ assays for biologically active triterpene glycosides from those plants closely related to Acacia victoriae, for example, members of the genus Acacia. These assays may make use of a variety of different formats and may depend on the kind of "activity" for which the screen is being conducted. Preferred assays comprise those directed to screening for anti-tumor activity, such as described herein for extracts from Acacia victoriae. As used herein, "anti-tumor activity" refers to the inhibition in tumor cells of cell-to-cell signaling, growth, metastasis, cell division, cell migration, soft agar colony formation, contact

inhibition, invasiveness, angiogenesis, tumor progression or other malignant phenotype or the induction of apoptosis. Particularly contemplated are functional assays which include measures of the use of the compounds of the invention as anti-fungal and anti-viral agents, piscicides or molluscicides, contaceptives, anthelmintics, UV-protectants, expectorants, diuretics, anti-inflammatory agents, regulators of cholesterol metabolism, cardiovascular effectors, anti-ulcer agents, analgesics, sedatives, immunomodulators, antipyretics, regulators of angiogenesis, and as agents for decreasing capillary fragility. Such assays will be well known to those of skill in the art in light of the instant disclosure. As well as in vitro and in vivo direct assays for activity, these assays may include measures of inhibition of binding to a substrate, ligand, receptor or other binding partner by a compound of the invention.

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Angiogenesis or neovascularization is a process by which cells are recruited by factor(s) produced by a tumor to provide the tumor with a nourishing vascular system. Inhibiting angiogenesis inhibits tumor expansion by limiting blood supply to the tumor. This function was examined using a bovine capillary endothelial cell proliferation assay on cells treated with Fraction 35 (UA-BRF-004-DELEP-F035). The assay was carried out as follows: bovine capillary endothelial cells were obtained and grown using standard procedures (Folkman et al., 1979). The cells were washed with PBS and dispersed in a 0.05% trypsin solution. A cell suspension (25,000 cells/ml) was made with DMEM+10% BCS+1% GPS, plated onto gelatinized 24 well culture plates (0.5 ml/well) and the suspension incubated for 24 h at 37.degree. C. The media was replaced with 0.25 ml of DMEM+5% BCS+1% GPS and different concentrations of UA-BRF-004-DELEP-F035 applied. After a 20 min incubation, media and bFGF were added to obtain a final volume of 0.5 ml DMEM+5% BCS+1% GPS+1 ng/ml bFGF. After 72 h the cells were dispersed in trypsin, resuspended in Hematall (Fischer Scientific, Pittsburg, Pa.) and counted by coulter counter (O'Reilly et al., 1997).

Detailed Description Text (569):
The results of the assay demonstrated significant inhibition of endothelial cell proliferation with or without basic fibroblast growth factor (FIG. 5). These results demonstrate that the active components of the plant extract are potent inhibitors of endothelial cell proliferation, which is often a predictor of in vivo suppression of angiogenesis. In addition, the fraction had no effect on migration of capillary endothelial cell, suggesting lack of toxicity to normal cells (FIG. 6).

Detailed Description Text (576):
In order to further elucidate the molecular targets of the active components of the Acacia victoriae plant extract, a study was conducted on the effect of F035 on phosphatidylinositol 3-kinase (PI3-kinase) activity, as well on AKT (protein kinase B, a serine-threonine kinase) activity, a downstream effector of PI3-kinase. P13-kinase is an enzyme which is implicated in growth factor signal transduction by associating with receptor and non-receptor tyrosine kinases. There are two known PI3-kinase inhibitors: wortmannin, a fungal metabolite, and LY294002, a synthetic compound which is structurally similar to the plant bioflavonoid quercetin.

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Detailed Description Text (578):
Next, the effect of F035 on AKT, a downstream effector of PI3-kinase, was studied. AKT, also known as protein kinase B, is a cellular homologue of viral oncogene v-AKT and is activated by number of growth factors and functions in a pathway involving PI3-K activation, which is sensitive to wortmannin. AKT codes for serine-threonine protein kinase, which has been shown to be

amplified in 12.1% of ovarian carcinomas and 2.8% of breast cancers. AKT is involved in an anti-apoptotic pathway through phosphorylation of Bad, an anti-apoptotic molecule. Ovarian cancer patients with AKT alterations appear to have poor prognosis (Bellacosa et al., 1995). AKT has been shown to actively block apoptosis, partly by activation of p70S6 kinase (Kennedy et al., 1997). p70S6 kinase is a mitogen activated serine-threonine protein kinase required for cell growth and G1 cell cycle progression (Chou and Blenis, 1996). The activity of p70S6 kinase is controlled by multiple phosphorylation events located within catalytic and pseudosubstrate region (Cheatham et al., 1995; Weng et al., 1995).

Detailed Description Text (579):
The effect of F035 on phosphorylation of AKT was analyzed as follows. Jurkat cells (5.times.10.sup.6) were serum starved and exposed to F035 for 15 h and 2 h with wortmannin at 37.degree. C. The cells were either induced with cd3XL (cd3 crosslink) or left uninduced for 10 min at 37.degree. C. and lysed in AKT lysis buffer and the proteins were resolved on 8% SDS-PAGE gels and analyzed by western-ECL using phospho-specific AKT (Ser 473; New England Biolabs) and total AKT antibody. An assay of the effect of F035 on p70S6 kinase can be carried out similarly, but using a Phosphoplus p70S6 kinase antibody kit (New England Biolabs) for analysis of p70S6 kinase (Ser 411, thr421/ser424) phosphorylation. The results of the AKT analysis (FIG. 22), demonstrated that cd3 crosslink induces phospho AKT slightly. Post treatment of cells with 1 and 2 .mu.g/ml of F035 caused a marked inhibition of AKT phosphorylation (active AKT), which is similar to a 2 h treatment of cells with 1 .mu.M of wortmannin. There was, however, no change in the expression of total AKT. Similar inhibition of AKT phosphorylation was also demonstrated using ovarian cancer cells OVCAR-3 and C-2 (HEY variant), and with Jurkat cells treated with 2-4 .mu.g/ml of F094. These findings demonstrate that F035 inhibits the phosphorylation of AKT in Jurkat cells and ovarian cancer cells. This is significant given that the PI3 kinase/AKT signaling pathway has been shown to deliver an anti-apoptotic signal (Kennedy et al., 1997). The results suggest F035 and F094 is mediating apoptosis of tumor cells through the suppression of the PI3-K signaling pathway.

Detailed Description Text (655):
The serum starved Jurkat cells were treated with 2 .mu.g/ml of F035 for 2-15 h or 0.5 h with wortmannin at 37.degree. C. PI 3-kinase activity was determined as described (Whitman et al., 1985; Royal and Park, 1995). PI3-kinase was immunoprecipitated from 1 mg of cellular protein using 5 .mu.l rabbit anti p85 antiserum at 4.degree. C. for 90 min. The immune complexes were collected on 20% protein A-sepharose beads for 90 min at 4.degree. C. Next the immunoprecipitates were resuspended in 30 .mu.l of kinase reaction buffer (33 mM Tris, pH 7.6, 125 mM NaCl, 15 mM MgCl.sub.2, 200 mM of adenosine, 20 mM ATP, 30 uCi [g-32P] adenosine triphosphate ATP). The PI3-kinase reaction was initiated by addition of 10 .mu.l of the PI suspension and 10 .mu.l of gamma-ATP and allowed to proceed for 30 min at room temp. Adding 100 .mu.l of 1 N HCl terminated the reaction. Lipids were extracted from the reaction mixture with chloroform: methanol (1:1) and resolved by thin layer chromatography (TLC) in chloroform: methanol: NH.sub.4 OH: H.sub.2 O (60:47:2:11.3) on silica gel G60 plates. Radio labeled phosphatidylinositol (PI) phosphate was visualized by autoradiography and inhibition was quantitated using a Storm 860 system (Molecular Dynamics).

Detailed Description Text (676):
To study the molecular target(s) of F035, the inventors investigated the PI3-kinase signaling pathway. The results of immunoprecipitation with anti-p85 antibody (adapter protein) probe and subsequent lipid kinase assay showed that F035 inhibits the activity of PI3-kinase in Jurkat cells. FIG. 45A demonstrates about 50-70% inhibition of PI3-kinase activity with in 2 hr post treatment with F035. By 6 hr 92-95% inhibition of PI3-kinase activity was observed which persisted up to 15 hr post treatment. Wortmannin [1 .mu.M, 30 min post treatment], a known PI3-kinase inhibitor showed similar inhibition of enzyme activity in Jurkat cells (FIG. 45A).

Detailed Description Text (679):
The inventors determined the effect of F035 and pure extracts on AKT, a serine threonine kinase and a downstream effector of the PI3-kinase signaling pathway. In contrast to the rapid inhibition of PI3-kinase activity, inhibition of AKT phosphorylation did not occur till 15 hr post treatment. Treatment of Jurkat cells with F035 (2 ml) for 15 hr led to decreased phosphorylation of AKT. However, this treatment also led to lowered levels of total AKT protein as can be seen in FIG. 45B. The inventors confirmed the inhibition of AKT activity with pure triterpene glycosides. Pure triterpene glycosides D1 & G1 (2 .mu.g/ml) also inhibited AKT phosphorylation and total AKT protein expression. (FIG. 45B). Treatment of Jurkat cells with LY 294002 and wortmannin (known PI3-kinase inhibitors) showed inhibition of AKT phosphorylation.

Detailed Description Text (682):
In order to further study the mediators of apoptotic pathway, the inventors evaluated the effects of F035, D1 and G1 on the transcription factor NF-.kappa.B which has been shown to be involved in apoptosis. The results in FIG. 46A show that in Jurkat cells, F035 inhibited the TNF-dependent activation of NF-.kappa.B in a dose dependent manner. Untreated cells and cells treated with F035 alone showed no activation of NF-.kappa.B. The inventors also confirmed these results with pure extracts D1 and G1. Pretreatment of cells with 2 ml of G1 and D1 resulted in 54% and 87% decrease in NF-.kappa.B levels respectively (FIG. 46B). Cells treated with D1 or G1 alone showed no activation of NF-.kappa.B (FIG. 4613). Since recently PI3-kinase has been shown to regulate NF-.kappa.B, pretreatment of cells with wortmannin (1 .mu.M) resulted in almost total inhibition of TNF-induced

NF-.kappa.B.

Detailed Description Text (685):
As the transcription of iNOS is regulated by NF-.kappa.B, the inventors investigated the effect of F035 on the induction of iNOS. In U-937 cells which were differentiated into macrophages the inventors induced iNOS in response to LPS (FIG. 46C). Pretreatment of these cells with F035 (1 .mu.g/ml) totally blocked the induction of iNOS. Wortmannin also had a similar effect on LPS induced iNOS in these cells.

Other Reference Publication (56):
Norman, "Studies on the mechanism of phosphatidylinositol 3-kinase inhibition by wortmannin and related analogs," J. Med. Chem., 39:1106-1111, 1996.

Other Reference Publication (58):
Okada et al., "Blockage of chemotactic peptide-induced stimulation of neutrophils by wortmannin as a result of selective inhibition of phosphatidylinositol 3-kinase," J. Bio. Chem., 269:3563-3567, 1994.

Your SELECT statement is:
s (wortmannin or ly294002) and (angiogenesis or cancer or tumor or neoplasm)

Items	File
766	5: Biosis Previews(R)_1969-2002/Dec W5
567	34: SciSearch(R) Cited Ref Sci_1990-2002/Dec W5
23	35: Dissertation Abs Online_1861-2003/Dec
1	65: Inside Conferences_1993-2003/Dec W5
456	71: ELSEVIER BIOBASE_1994-2002/Dec W5
625	73: EMBASE_1974-2003/Dec W5
26	94: JICST-EPlus_1985-2002/Oct W3
19	98: General Sci Abs/Full-Text_1984-2002/Nov
10	135: NewsRx Weekly Reports_1995-2003/Dec W5
196	144: Pascal_1973-2002/Dec W4
56	149: TGG Health&Wellness DB(SM) 1976-2002/Dec W3
841	155: MEDLINE(R)_1966-2002/Dec W3
178	156: ToxFile_1965-2002/Nov W3
760	159: Cancerlit_1975-2002/Oct
4	162: CAB Health_1983-2002/Nov
26	172: EMBASE Alert_2002/Dec W5
14	266: FEDRIP_2002/Nov
8	370: Science_1996-1999/Jul W3
38	399: CA SEARCH(R)_1967-2003/UD=13801
1	442: AMA Journals_1982-2003/Jan B2

Set	Items	Description
S1	13158	WORTMANNIN OR LY295002
S2	5473	LY294002
S3	237	(S1 OR S2) AND ANGIOGENE?
S4	64	S3 AND CANCER
S5	35	S4 NOT PY=>2001
S6	15	RD (unique items)

6/9/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12698814 BIOSIS NO.: 200000452316
Angiopoietin-2 at high concentration can enhance endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway.
AUTHOR: Kim Injune; Kim Ji-Hye; Moon Sang-Ok; Kwak Hee Jin; Kim Nam-Gyun; Koh Gou Young(a)
AUTHOR ADDRESS: (a)National Creative Research Initiatives Center for Cardiac Regeneration, Institute of Cardiovascular Research, Chonbuk University School of Medicine, San 2-20, Keum-Am-Dong, Chonju, 560-180** South Korea
JOURNAL: Oncogene 19 (39):p4549-4552 14 September, 2000
MEDIUM: print
ISSN: 0950-9232
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: The angiopoietin-Tie2 system in endothelial cells is an important regulator of vasculogenesis and vascular integrity. High levels of angiopoietin-2 (Ang2) mRNA are observed in vascular activation during tumorigenesis. Although Ang2 is known to be a naturally occurring antagonist of angiopoietin-1 (Ang1) in vivo, the exact function of Ang2 itself is not known. Here, we found that a high concentration of Ang2 (800 ng/ml) acts as an apoptosis survival factor for endothelial cells during serum deprivation apoptosis. The survival effect of high concentration Ang2 was blocked by pre-treatment with soluble Tie2 receptor and the PI 3'-kinase-specific inhibitors, **wortmannin** and **LY294002** . Accordingly, 800 ng/ml of Ang2 induced phosphorylation of Tie2, the p85 subunit of phosphatidylinositol 3'-kinase (PI 3'-kinase), and serine-threonine kinase Akt at Ser473 in the human umbilical vein endothelial cells; lower concentrations of Ang2 (50-400 ng/ml) did not

produce notable effects. These findings indicate that at high concentrations, Ang2, like Ang1, can be an apoptosis survival factor for endothelial cells through the activation of the Tie2 receptor, PI 3'-kinase and Akt, and thus may be a positive regulator of tumor **angiogenesis** .

6/9/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12643864 BIOSIS NO.: 200000397366
Vascular endothelial growth factor up-regulates ICAM-1 expression via the phosphatidylinositol 3 OH-kinase/AKT/nitric oxide pathway and modulates migration of brain microvascular endothelial cells.
AUTHOR: Radisavljevic Zivotije; Avraham Hava; Avraham Shalom(a)
AUTHOR ADDRESS: (a)Division of Experimental Medicine, Beth Israel Deaconess Medical Center, Harvard Inst. of Medicine, 4 Blackfan Circle, Boston, MA, 02115**USA
JOURNAL: Journal of Biological Chemistry 275 (27):p20770-20774 July 7, 2000
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Endothelium of the cerebral blood microvessels, which constitutes the major component of the blood-brain barrier, controls leukocyte and metastatic **cancer** cell adhesion and trafficking into the brain parenchyma. In this study, using rat primary brain microvascular endothelial cells (BMEC), we demonstrate that the vascular endothelial growth factor (VEGF), a potent promoter of **angiogenesis** , up-regulates the expression of the intracellular adhesion molecule-1 (ICAM-1) through a novel pathway that includes phosphatidylinositol 3 OH-kinase (PI3K), AKT, and nitric oxide (NO), resulting in the migration of BMEC. Upon VEGF treatment, AKT is phosphorylated in a PI3K-dependent manner. AKT activation leads to NO production and release and activation-deficient AKT attenuates NO production stimulated by VEGF. Transfection of the constitutive myr-AKT construct significantly increased basal NO release in BMEC. In these cells, VEGF and the endothelium-derived NO synergistically up-regulated the expression of ICAM-1, which was mediated by the PI3K pathway. This activity was blocked by the PI3K-specific inhibitor, **wortmannin** . Furthermore, VEGF and NO significantly increased BMEC migration, which was mediated by the up-regulation of ICAM-1 expression and was dependent on the integrity of the PI3K/AKT/NO pathway. This effect was abolished by **wortmannin** , by the specific ICAM-1 antibody, by the specific inhibitor of NO synthase, NG-L-monomethyl-arginine (L-NMMA) or by a combination of **wortmannin** , ICAM-1 antibody, and L-NMMA. These findings demonstrate that the angiogenic factor VEGF up-regulates ICAM-1 expression and signals to ICAM-1 as an effector molecule through the PI3K/AKT/NO pathway, which leads to brain microvessel endothelial cell migration. These observations may contribute to a better understanding of BMEC **angiogenesis** and the physiological as well as pathophysiological function of the blood-brain barrier, whose integrity is crucial for normal brain function.

6/9/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12435110 BIOSIS NO.: 200000188612
Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer **cells: Implications for tumor** angiogenesis **and therapeutics.**
AUTHOR: Zhong Hua; Chiles Kelly; Feldser David; Laughner Erik; Hanrahan Colleen; Georgescu Maria-Magdalena; Simons Jonathan W(a); Semenza Gregg L
AUTHOR ADDRESS: (a)Brady Urological Institute, Marburg 409, Johns Hopkins

Hospital, 600 North Wolfe Street, Baltimore, MD, 21287-2411**USA
JOURNAL: Cancer Research 60 (6):p1541-1545 March 15, 2000 order
ISSN: 0008-5472
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Dysregulated signal transduction from receptor tyrosine kinases to phosphatidylinositol 3-kinase (P13K), AKT (protein kinase B), and its effector FKBP-rapamycin-associated protein (FRAP) occurs via autocrine stimulation or inactivation of the tumor suppressor PTEN in many cancers. Here we demonstrate that in human prostate **cancer** cells, basal-, growth factor-, and mitogen-induced expression of hypoxia-inducible factor 1 (HIF-1) alpha, the regulated subunit of the transcription factor HIF-1, is blocked by **LY294002** and rapamycin, inhibitors of P13K and FRAP, respectively. HIF-1-dependent gene transcription is blocked by dominant-negative AKT or P13K and by wild-type PTEN, whereas transcription is stimulated by constitutively active AKT or dominant-negative PTEN. **LY294002** and rapamycin also inhibit growth factor- and mitogen-induced secretion of vascular endothelial growth factor, the product of a known HIF-1 target gene, thus linking the PI3K/PTEN/AKT/FRAP pathway, HIF-1, and tumor **angiogenesis** . These data indicate that pharmacological agents that target P13K, AKT, or FRAP in tumor cells inhibit HIF-1alpha expression and that such inhibition may contribute to therapeutic efficacy.

REGISTRY NUMBERS: 154447-36-6: **LY294002** ; 62229-50-9: EPIDERMAL GROWTH FACTOR; 115926-52-8: PHOSPHATIDYLINOSITOL 3-KINASE; 148640-14-6: PROTEIN KINASE B; 53123-88-9: RAPAMYCIN; 127464-60-2: VASCULAR ENDOTHELIAL GROWTH FACTOR
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Tumor Biology
BIOSYSTEMATIC NAMES: Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: DU145 cell line (Hominidae); PC-3 cell line (Hominidae); PPC-1 cell line (Hominidae); TSU cell line (Hominidae)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): Animals; Chordates; Humans; Mammals; Primates; Vertebrates
DISEASES: prostate **cancer** --neoplastic disease, reproductive system disease/male, treatment, urologic disease
CHEMICALS & BIOCHEMICALS: FKBP-rapamycin-associated protein; **LY294002** --PI3K inhibitor; PTEN--tumor suppressor; epidermal growth factor; hypoxia-inducible factor 1-alpha--expression; phosphatidylinositol 3-kinase; protein kinase B; rapamycin--FRAP inhibitor; vascular endothelial growth factor--secretion
MISCELLANEOUS TERMS: signal transduction--autocrine stimulation; tumor **angiogenesis**

6/9/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10762758 BIOSIS NO.: 199799383903
Potent inhibition of angiogenesis **by** wortmannin **, a fungal metabolite.**
AUTHOR: Oikawa Tsutomu(a); Shimamura Mariko
AUTHOR ADDRESS: (a)Dep. Cancer Therapeutics, Tokyo Metropolitan Inst. Med. Sci., 3-18-22 Honkomagome, Bunkyo-ku, To**Japan
JOURNAL: European Journal of Pharmacology 318 (1):p93-96 1996
ISSN: 0014-2999
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: **Wortmannin** ((1S-(1-alpha,6b-alpha,9-alpha-beta,11-alpha,11b-beta))-11-(acetyloxy)-1,6b,7,8,9-alpha,10,11,11b-octahydro-1-(methoxymethyl)-9a,11b-dimethyl3H-furo(4,3,2-de)indeno(4,5-h)-2-benzopyran-3,6,9-trione), a fungal metabolite that is as a selective inhibitor of phosphatidylinositol 3-kinase, was evaluated for its potential as an inhibitor of in vivo **angiogenesis** in a bioassay system involving growing chick embryo chorioallantoic

membranes. It showed dose-dependent inhibitory activity against embryonic **angiogenesis** . This inhibition occurred at a dose as low as 1 ng (2.3 pmol) per egg and the ID-50 value was 30 ng/egg. These findings suggest that **wortmannin** is a new **angiogenesis** inhibitor, and that it may be a lead antibiotic for a novel class of therapeutic agents for **angiogenesis** -dependent diseases like **cancer** , diabetic retinopathy and rheumatoid arthritis.

REGISTRY NUMBERS: 19545-26-7: **WORTMANNIN** ; 115926-52-8: PHOSPHATIDYLINOSITOL 3-KINASE
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Cardiovascular System (Transport and Circulation); Development; Enzymology (Biochemistry and Molecular Biophysics); Pharmacognosy (Pharmacology); Pharmacology
BIOSYSTEMATIC NAMES: Galliformes--Aves, Vertebrata, Chordata, Animalia
ORGANISMS: chicken (Galliformes)
BIOSYSTEMATIC CLASSIFICATION (SUPER TAXA): animals; birds; chordates; nonhuman vertebrates; vertebrates
CHEMICALS & BIOCHEMICALS: **WORTMANNIN** ; PHOSPHATIDYLINOSITOL 3-KINASE
MISCELLANEOUS TERMS: Research Article; ANTI-ANGIOGENIC ACTIVITY; DEVELOPMENT; EMBRYO; EMBRYONIC **ANGIOGENESIS** ; ENZYME INHIBITOR; FUNGAL METABOLITE; PHARMACODYNAMICS; PHARMACOLOGY; PHOSPHATIDYLINOSITOL 3-KINASE; **WORTMANNIN**

6/9/7 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

08449753 Genuine Article#: 285VU Number of References: 44
Title: Phosphatidylinositol 3-kinase signaling mediates angiogenesis **and expression of vascular endothelial growth factor in endothelial cells**
Author(s): Jiang BH; Zheng JZ; Aoki M; Vogt PK (REPRINT)
Corporate Source: SCRIPPS CLIN & RES INST,DEPT MOL & EXPT MED, BCC239, 10550 N TORREY PINES RD/LA JOLLA//CA/92037 (REPRINT); SCRIPPS CLIN & RES INST,DEPT MOL & EXPT MED/LA JOLLA//CA/92037
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 2000, V97, N4 (FEB 15), P1749-1753
ISSN: 0027-8424 Publication date: 20000215
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418
Language: English Document Type: ARTICLE
Geographic Location: USA
Subfile: CC LIFE--Current Contents, Life Sciences;
Journal Subject Category: MULTIDISCIPLINARY SCIENCES
Abstract: Phosphatidylinositol 3-kinase (PI 3-kinase) is a signaling molecule that controls numerous cellular properties and activities. The oncogene v-p3k is a homolog of the gene coding for the catalytic subunit of PI 3-kinase, p110 alpha. P3k induces transformation of cells in culture, formation of hemangiosarcomas in young chickens, and myogenic differentiation in myoblasts. Here, we describe a role of PI 3-kinase in **angiogenesis** . Overexpression of the v-P3k protein or of cellular PI 3-kinase equipped with a myristylation signal, Myr-P3k, can induce **angiogenesis** in the chorioallantoic membrane (CAM) of the chicken embryo. This process is characterized by extensive sprouting of new blood vessels and enlargement of preexisting vessels. Overexpression of the myristylated form of the PI 3-kinase target Akt, Myr-Akt, also induces **angiogenesis** . Overexpression of the tumor suppressor PTEN or of dominant-negative constructs of PI 3-kinase inhibits **angiogenesis** in the yolk sac of chicken embryos, suggesting that PI 3-kinase and Akt signaling is required for normal embryonal **angiogenesis** . The levels of mRNA for vascular endothelial growth factor (VEGF) are elevated in cells expressing activated PI 3-kinase or Myr-Akt. VEGF mRNA levels are also increased by insulin treatment through the PI 3-kinase-dependent pathway. VEGF mRNA levels are decreased in cells treated with the PI 3-kinase inhibitor **LY294002** and restored by overexpression of v-P3k or Myr-Akt, Overexpression of VEGF by the RCAS vector induces **angiogenesis** in chicken embryos. These results suggest that PI 3-kinase plays an important role in **angiogenesis** and regulates VEGF expression.

6/9/13 (Item 3 from file: 73)
DIALOG(R)File 73:EMBASE

10583421 EMBASE No: 2000048228

Oncogenes and tumor angiogenesis **: Differential modes of vascular endothelial growth factor up-regulation in ras-transformed epithelial cells and fibroblasts**

Rak J.; Mitsuhashi Y.; Sheehan C.; Tamir A.; Viloria-Petit A.; Filmus J.; Mansour S.J.; Ahn N.G.; Kerbel R.S.
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Cancer Research (CANCER RES.) (United States) 15 JAN 2000, 60/2 (490-498)
CODEN: CNREA ISSN: 0008-5472
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 71

A possible link between oncogenes and tumor **angiogenesis** has been implicated by the finding that expression of various oncogenes, particularly mutant ras, can lead to a marked induction of a potent paracrine stimulator of **angiogenesis** , vascular endothelial growth factor (VEGF). We sought to determine how oncogenic ras induction of VEGF is mediated at the molecular level and whether the mechanisms involved differ fundamentally between transformed epithelial cells and fibroblasts. Our results suggest that in a subline (called RAS-3) of immortalized nontumorigenic rat intestinal epithelial cells (IEC-18) that acquired a tumorigenic phenotype upon transfection of mutant ras, up-regulation of VEGF occurs in the absence of an autocrine growth factor circuit. The expression of VEGF mRNA and protein by RAS-3 cells was strongly suppressed in the presence of **LY294002** , an inhibitor of phosphatidylinositol 3'-kinase, but remained largely unaffected in the same cells treated with an inhibitor (PD98059) of mitogen-activated protein/extracellular signal-regulated kinase kinase 1 (MKK/MEK-1). This is consistent with the observation that overexpression of a constitutively activated mutant of MEK-1 (DeltaN3/S222D) in the parental IEC-18 cells did not result in up-regulation of VEGF production. The impact of mutant ras on VEGF expression was also significantly amplified at high cell density, conditions under which RAS-3 cells became less sensitive to **LY294002** -induced VEGF downregulation. In marked contrast to cells of epithelial origin, ras- transformed murine fibroblasts (3T3RAS) up-regulated VEGF in a manner that was strongly inhibitable by MEK-1 blockade (i.e. treatment with PD98059), whereas these cells were relatively unaffected by treatment with the phosphatidylinositol 3'-kinase inhibitor • **LY294002** . In addition, VEGF was upregulated by 2-3-fold in NIH3T3 cells overexpressing mutant MEK-1. Collectively, the data suggest that the stimulatory effect of mutant ras on VEGF expression is executed in a nonautocrine and cell type-dependent manner: and that it can be significantly exacerbated by physiological/environmental influences such as high cell density.

1: Clin Cancer Res 2002 Jun;8(6):1957-63

The in vitro and in vivo effects of 2-(4-morpholinyl)-8-phenyl-chromone (LY294002), a specific inhibitor of phosphatidylinositol 3'-kinase, in human colon cancer cells.

Semba S, Itoh N, Ito M, Harada M, Yamakawa M.

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PURPOSE: Phosphatidylinositol 3'-kinase (PI3K) and Akt/protein kinase B(PKB) allow for escape from apoptosis in various human cancer cells. We postulated that 2-(4-morpholinyl)-8-phenyl-chromone (LY294002), a PI3K inhibitor, should inactivate Akt/PKB, consequently inhibiting cell proliferation and inducing apoptosis in vitro and in vivo. EXPERIMENTAL DESIGN: Human colon cancer cell lines (DLD-1, LoVo, HCT15, and Colo205) and their mouse xenografts (DLD-1 and LoVo) were used in this study. The expression of phosphorylated Akt (Ser(473)) and apoptosis in cancer cells were determined by immunoblotting and immunohistochemistry. To evaluate the activity of caspase-3 in culturing cells, the caspase colorimetric assay was also performed. RESULTS: LY294002 demonstrated a remarkable growth-inhibitory and apoptosis-inducing effect in these colon cancer cell lines, with decreased expression of phosphorylated Akt (Ser(473)). However, there was a great discrepancy between the sensitivity for LY294002 and the level of expression of phosphorylated Akt. Although the LoVo and Colo205 cells exhibited high sensitivity to LY294002 with increased apoptosis, the DLD-1 and HCT15 cells did not show rapid induction of apoptosis. The caspase-3 activity was significantly high in the LoVo cells but not in the DLD-1 cells.

In the experiments using mouse xenografts, we found that LY294002 administration in vivo also resulted in suppression of tumor growth and induction of apoptosis, especially in the LoVo tumors, and therefore showed remarkable effectiveness in the mouse peritonitis carcinomatosa model. CONCLUSIONS: PI3K-Akt/PKB plays an important role in colon cancer development and progression by helping to promote cell growth and allowing cells to escape apoptosis. These results propose the usefulness of LY294002 as an antitumoral agent for patients with colorectal cancer.

PMID: 12060641 [PubMed - indexed for MEDLINE]
